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EFFECT OF GROWTH AGE PERIOD ON BIOCHEMICAL COMPOSITION OF *PLANTAGO MAJOR* PLANT

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ABSTRACT

Objectives: The present study was aimed to find the effect of growth age period of *Plantago major* leaves on the biochemical composition of the plant.

Methods: Leaves of *P. major* have been assessed for it is total phenolic, total flavonoid and total tannin contents at different growth age period (vegetative and generative).

Results: revealed significant differences in biochemical compositions between two growth age periods of the plant, generative leaves exhibited significant amount of flavonoid (0.12±0.002 mg QE\g DW) and tannin contents (0.12±0.002 mg GAE\g DW) while a significant total phenolic contents were expressed by vegetative leaves (0.015±0.001 mg GAE\ g DW).

Conclusions: The research findings emphasized great effect of growth age period on biochemical composition of the *P. major* leaves.

Key Words: Vegetative period, Generative period, Total phenolic content, Total flavonoid, Total tannin content

INTRODUCTION

Mankind has always been screened for agents to treat diseases since aliments were as old as life itself. Disease eradication has been performed by the usage of herbal remedies and medicinal plants. Everyday there were discovering of new medicinal plants. There collection must be at right season and specific growth stage for obtaining an optimized quantity of bioactive constituents [1]. Extracted phytochemical compounds from plant source are phenols, alkaloids, tannin, saponin, flavonoids and lignin which exert biological activity either as prophylactic or treating agents of various diseases such as diabetes, cancer, heart diseases and high blood pressure [2]. Recently, phytochemicals made a valuable venue of research in medical and food industry to emphasize their biological activities [3]. Phytochemical contents of plant affected by various factors. These factors comprised environmental conditions, season, plant age, growth factors and leaf maturity. The biological activity of medicinal plants changes with the respect to the plant age. Moreover, the right authenticated plant part at specified age period should be harvested in selected season before introducing the plant for the drug manufacturing process, to optimize the herbal preparation potency[4-7].

Plantago major L. is perennial herb belong to Plantaginaceae family, grows about 15 cm in height with variant size. The leaves were in rosettes having elliptical to ovate shape. The flowers are brownish-green color, small size appear on long non-ramified spikes [9]. *P. major* commonly known as a weed only while traditional medicine identified it is value as a medicinal plant. The plant mostly known for its therapeutic activity in wound healing properties [9,10]. Traditionally plant attributed in a number of disease curing processes distributed in worldwide like, infectious diseases, problems concerning the digestive organs, reproduction, against tumours, pain relieving, fever reducing, skin diseases, respiratory organs and the circulation [11-15]. The plant contains a number of medicinal active constituents such as phenolic compounds, flavonoids, alkaloids, irodiod glycoside, carbohydrate, lipid, vitamins and coumarin [12-22].

The present study was aimed to find the effect of different age growth period of *Plantago major* plant grown naturally

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in Erbil city on the concentration of phytochemical more specifically total phenolic, total flavonoid and total tannin content in ethanolic extract of plant.

MATERIAL AND METHODS

Plant material collection:

Leaves of *Plantago major* plant have been harvested at two different growth age period vegetative and generative growth periods, authenticated in Pharmacognosy Department, Pharmacy College\Hawler Medical University. Plant parts dried in shade, kept in close container at 25 ^{0}C.

Assessment of Biochemical composition:

Plant parts have been assessed for it is biochemical composition by estimation of total phenolic, total flavonoid and total tannin contents.

Estimation of total phenol content:

Total phenol compounds have been estimated according to the Folin-Ciocalteu method with slight modifications [23]. Briefly, 1ml extract prepared from (0.5g) of crude plant material was mixed with 9 ml of distilled water. One ml of Folin-Ciocalteu phenol reagent was added to the mixture. The mixture mixed and allowed to stand for 5 minute at room temperature, then 10ml of (7%) sodium carbonate were added. The volume have been adjusted to 25ml and incubated for 90 minutes at room temperature. The absorbance was measured at 750nm using UV visible spectrophotometer. Total phenol content were estimated from calibration curve obtained from measuring the absorbance of standard concentration of gallic acid solution in distilled water with concentrations [20, 40, 60, 80 and 100 mcg\ml]. The results were expressed as mg of gallic acid equivalent (GAE)\gram of dry powdered plant material (DW).

Estimation of total flavonoid content:

Total flavonoid content was measured by the aluminium chloride colorimetric method [24]. Aliquot of 1 ml extract prepared from 1g of powdered plant material, was added to 10 ml volumetric flask containing 4 ml of distilled water. About 0.3 ml sodium carbonate 5% was added to the flask and after 5 min, 0.3 ml aluminium chloride (10%) was added. Two ml sodium hydroxide (1 M) was added at 6th min and the total volume was adjusted up to 10 ml with distilled water. The solution was mixed thoroughly and the absorbance level was determined at 510 nm using UV visible spectrophotometer. The total flavonoid content was measured from calibration curve obtained from measuring absorbance of standard concentration of querstine solution in ethanol (80%) with concentrations [20, 40, 60, 80 and 100 mcg\ml] The results was expressed as mg of querstine equivalents (QE)\ gram plant dry weight material (DW).

Estimation of total tannin content:

Total tannin content have been estimated according to the Folin-Ciocalteu method described by Tamilselvi *et al*, 2012 [25] with slight modifications. A volume of 0.1 ml of the plant material extract prepared from (0.5g) crude drug material was added to 7.5 ml of distilled water and (0.5 ml) of Folin-Ciocalteu Phenol reagent, to the mixture (1ml) of (35%) sodium carbonate solution was added. Using distilled water the volume has been adjusted to (10ml). The mixture was shaken and incubated for 30 min at room temperature and absorbance was read at 725 nm. Total tannin content were measured from calibration curve obtained from measuring absorbance of standard concentrations of gallic acid solution in distilled water with concentrations [20, 40, 60, 100 mcg of gallic acid\ml]. The results of tannin content were expressed in terms of mg of gallic acid equivalent (GAE)\ gram of plant dry weight material (DW).

Statistical analysis:

All data were collected from triplicate procedure works expressed as mean ± standard deviation (SD). Two way ANOVA method used for comparison between means considering (p value < 0.0001) statistically significant.

RESULTS

Plantago major plant have been evaluated at two different growth age period (generative and vegetative) periods for it biochemical composition using standard curves of gallic acid for total phenolic, querstine for total flavonoid and gallic acid for total tannin contents (figure.1, figure.2 and figure.3). Significant total phenolic detected in vegetative growth age period while significant total flavonoid and total tannin contents were detected in generative growth age period (p value < 0.0001) (Table.1).

DISCUSSION

Plantago major leaves is a medicinal plant used by local communities in treatment of variant diseases grown naturally in different places of Erbil city, have been assessed for their biochemical composition at different growth age periods (vegetative and generative periods), since age growth period affect phytochemical concentration in plant [4-7]. The total phenolic content of *Plantago major* leaves were estimated from the standard curve equation ($y=0.08x - 0.120$, $r^2 = 0.9$) shown in figure.1. A significant total phenolic content were exhibited by the vegetative period leaves ($p < 0.0001$) in comparison to the total phenol expressed by the generative

period leaves [Table .1], the finding were consistent with the finding of the Achakzai *et al.*2009 [6], which confirmed the low levels of phenol in young leaves of *Rhododendron* spp., since the plant utilized the phenol for the primary metabolic process required for plant growth.

A significant amount of flavonoid content [Table.1.] were estimated in generative period of *P. major* leaves ($p < 0.0001$) from standard curve equation ($y=0.007x+0.036$, $r^2 = 0.98$) shown in figure .2. Our study results were compatible to the findings of Miean & Mohamed, 2001 [26] and Albach et al, 1981 [27] and in agreement to the records of Behn *et al*, 2011[28] who reported high flavonoid contents in generative period leaves of lettuce, while the results were in contrast to the finding of Ali, *et al* 2014 [29] which reported low level of flavonoid in older leaves (generative period) in correspondence to the younger ones.

Similarly to the flavonoid content, tannin contents were showed upsurge with increasing of plant age. The exhibited tannin contents of plant in different age growth periods showed significant variation in tannin contents ($p < 0.0001$), since generative period leaves expressed higher tannin contents [Table.1.] which have been estimated from standard curve equation ($y=0.001x-0.012$, $r^2=0.996$) shown in figure .3. Plant growth age period relation with the chemical compositions of the plant have been confirmed by Farias, 2003 [30], Esmelindro *et al*, 2004 [31].

Generally the chemical composition varies according to the growth age period of and it is requirements for growth. *P. major* leaves expressed high contents of two of evaluated phytochemical constituents flavonoid and tannin contents in generative period while the total phenolic compounds showed high values at vegetative periods. Further research have been recommended to evaluate the plant from biological activity points to standardized the medicinal age growth period of the plant.

CONCLUSION

From study results we concluded that plant growth age period reflected on the biochemical composition of the medicinal plants. Plant phytochemicals concentration either increase or decrease according age growth period of plant, choosing the right period for plant harvesting is very important for the medicinal value of the herbal preparation.

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Conflict of interest:

There was no any conflict of interest to be declared by the author.

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Table 1: Total phenol, total flavonoid and total tannin content of Plantago major at different age growth period:

Age growth period	Total phenol* (mg GAE\g DW)	Total flavonoid* (mg QE\g DW)	Total tannin* (mg GAE\g DW)
Vegetative period	0.015±0.001[a]	0.095±0.003	0.151±0.002
Generative period	0.013±0.002	0.12±0.002[a]	0.205±0.004[a]
P value	<0.0001	<0.0001	<0.0001

*Data are means ±SD, (n=3).
[a]statistically express higher value.

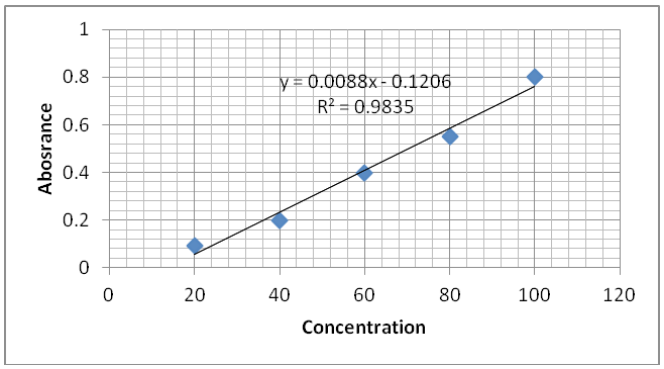

Figure 1: Calibration curve of Gallic acid for total phenol estimation

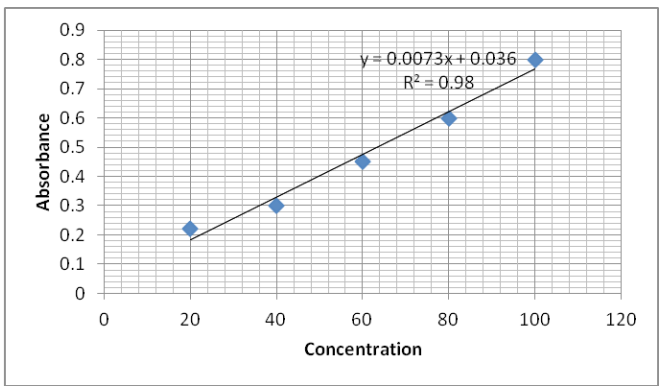

Figure 2: Calibration curve of Querstine for total flavonoid estimation

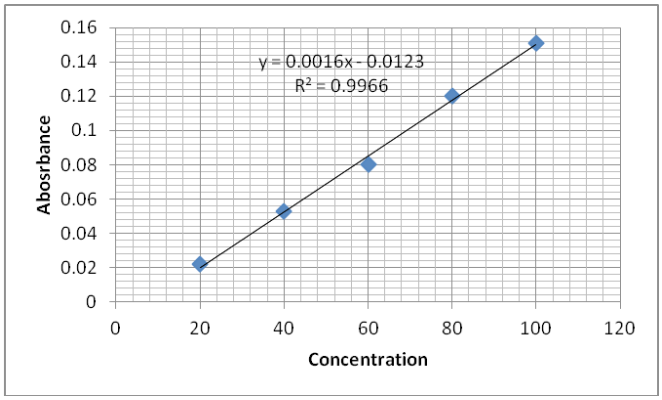

Figure 3: Calibration curve of Gallic acid for tannin content estimation